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CONTRACTING ORGANIZATION: University of Missouri
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13. ABSTRACT ***(Maximum 200 Words)***

Our understanding of the molecular genetic mechanisms of prostate carcinogenesis is still primitive. Due to this, effective preventive and therapeutic approaches to this prevalent disease have not been obtained yet. Our research goal is to use mouse molecular genetic approaches to examine the potential roles of Bone Morphogenetic Protein (BMP) signaling pathway in prostate epithelial cell growth and carcinogenesis. To accomplish this, we have designed a potent artificial prostate-specific promoter to drive constitutively active and dominant negative BMP receptors and constitutively active SMAD1 (a down stream signaling protein of BMP family) in the mouse. The major work of DNA constructs has been finished and transgenic mice will soon be generated for further characterization.

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Introduction

There is increasing amount evidence supporting the roles of Bone Morphogenetic Proteins (BMP) in cell proliferation, differentiation, and survival. BMP signaling components (BMP4, BMP7, BMP receptors, and their downstream signal transducers) are expressed in the adult prostate epithelium of the mouse. The objective of our research is to investigate the possible roles of BMP signaling transduction in the proliferation of prostate epithelium and the tumorigenesis of prostate epithelial cells. To accomplish this goal, we have integrated our current knowledge in prostate-specific gene expression, BMP signaling, and transgenic mice and designed a potentially very potent prostate-specific and androgen-dependent artificial promoter to drive gene expression in prostate epithelium.

Body

Although our research on this project during the first year has not resulted in any publication, the new development of a potentially powerful expression vector to drive high levels of gene expression in the prostate may prove to be very valuable to the field of prostate cancer research. New studies on the rat probasin promoter (PB) by Dr. Robert Matusik's group at Vanderbilt University indicated that to use the initial PB promoter driving transgene expression in the prostate yields a low percentage of high-level expressing lines. Therefore, it will take considerable amount of work to generate enough transgenic mouse lines before a useful mouse line can be obtained. In order to reduce the load of future works and more importantly to obtain a more powerful promoter for prostate expression, set forth to construct an artificial prostate promoter (PBI) as shown in Figure 1. Therefore, our plan for the first has been slightly modified. However, since this modification will expedite our work in the second year, it will improve the overall quality of our research and no changes in our overall plan are anticipated at this point.

In the new vector pPBI, several critical features have been added to enhance prostate-specific and androgen-dependent expression. First, the original PB promoter (426 bp) is present with all its components including TATA box. Therefore, PBI should be at least as potent as the original PB promoter. Second, a region of 340-bp 5' to the minimal PB promoter (containing two copies of the androgen response element (ARE) is linked to additional 3 copies of ARE to enhance the androgen response. Moreover, this whole region with 5 copies of ARE is repeated 5 times. Therefore, PBI contains the PB promoter and 25 copies instead of 2 copies of ARE. Thirdly, an artificial intron, a multiple cloning site (MCS), and an SV40 late poly (A) signal are linked 3' to the PB promoter to increase the transcription efficiency and to facilitate the insertion of different cDNAs.

It took several months to construct pPBI. Since then we have successfully inserted cDNAs for the CA-BMPRIA and DN-BMPRIA into pPBI vector and we are generating transgenic mice with these new DNA constructs. We will finish the construction CA-SMAD1 within a few weeks for transgenic mice generation. With the above modification, we hope to obtain necessary transgenic mouse lines with much reduced workloads. Furthermore, after we have a complete investigation, pPBI vector will be available to other researchers in the field of prostate cancer research.

[redacted]

In conclusion, in order to improve the efficiency of prostate gene expression and to expedite our research, we have constructed a potentially more powerful vector pPBI. pPBI serves as a parental vector for CA-BMPRIA, DN-BMPRIA, and CA-SMAD1 transgenic constructs. These constructs will allow us to generate necessary transgenic mice for our research with minimal amount of workloads. No changes for our overall research goal are anticipated.

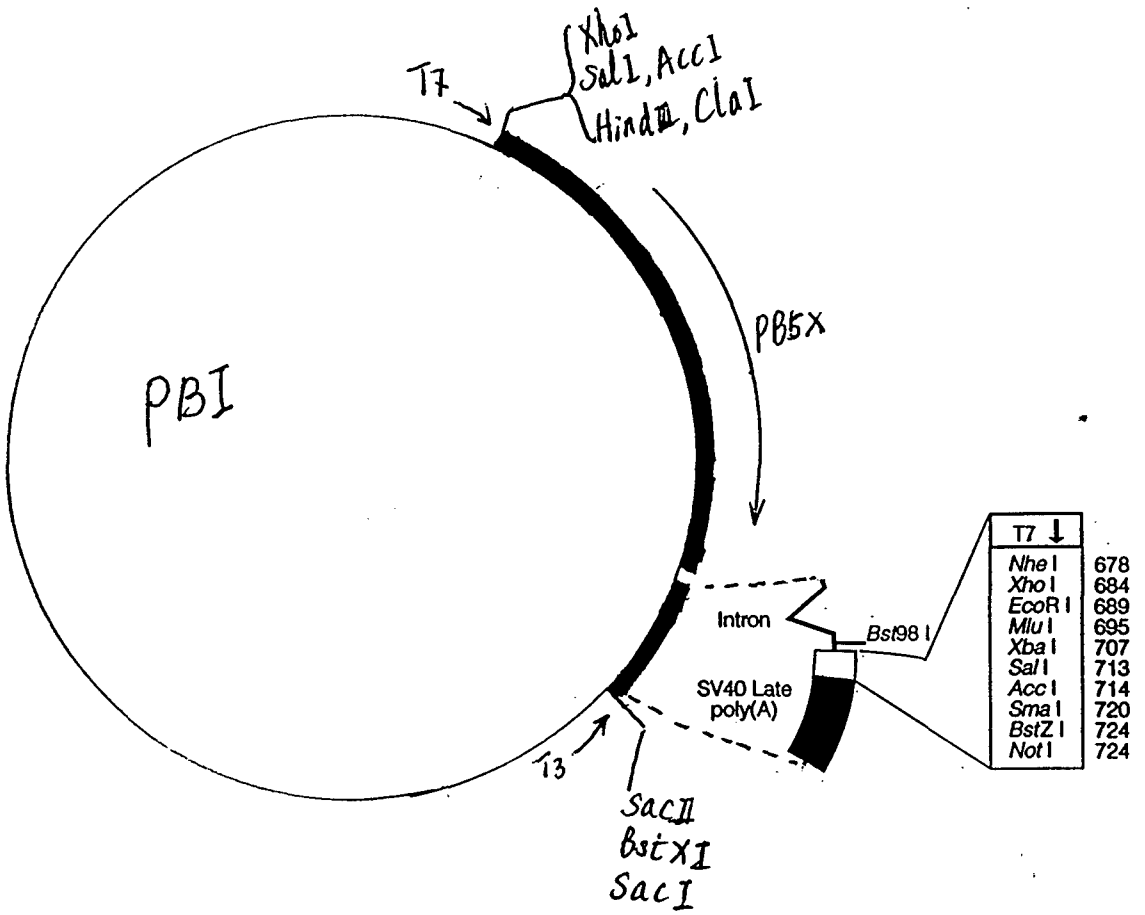

Figure 1. Schematic representation of pPBI vector. The backbone of this vector is derived from PCRscript (Strategene). The promoter region (PB5X) contains 25 copies of androgen responsive element, 5 copies of rat probasin (PB) promoter sequence without TATA box, and a full 426 bp PB promoter. An intron followed by a multiple cloning sites (MCS) and an SV 40 late poly (A) signal (derived from pSI vector, Promega) are inserted downstream of PB promoter. Therefore, different cDNAs can be easily cloned into the MCS. Various restriction enzyme digestion sites and the direction of T3 and T7 promoters are indicated.

DEPARTMENT OF THE ARMY
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